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16. Abstracts

This report describes studies conducted to determine if soils and vegetation could serve as a sink for atmospheric carbon monoxide. This was accomplished by exposing various soils and plant samples to experimental atmospheres containg carbon monoxide. The results show (1) Higher plants tested had no detectable capacity for decreasing carbon monoxide levels in the atmosphere surrounding them; (2) Soils from a wide range of ecotypes depleted carbon monoxide from atmospheres above them at rates averaging 8.44 $m'g/hr/m^2$ of soil surface; (3) The depletion of carbon monoxide by soils was sensitive to temperature, with a maximum depletion rate at 30° C and a Q_{10} of 3-6; (4) Constant and prolonged exposure of soil to carbon monoxide reduced the soil's microflora population and also reduced the soil's capacity to deplete carbon monoxide from the atmosphere; (5) Bacteria and fungi isolated from soils; (6) Preliminary experiments with C^{14}-labeled carbon monoxide indicated that soils oxidize carbon monoxide to carbon monoxide,

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I INTRODUCTION

The increasing emission of carbon monoxide (CO) through the burning of fossil fuels, notably gasoline by motor vehicles, has caused serious concern among public health officials and representatives of the automotive and petroleum industries. It has been estimated that over 200 million metric tons of CO per year are liberated into the earth's atmosphere due to man's activities alone (18). Yet, ambient concentrations do not appear to have changed appreciably as a result, in spite of calculations (14) showing that at this rate of liberation, the ambient CO level should double within 4-5 years. The fate of CO liberated into the atmosphere therefore has aroused scientific curiosity and become medically significant. The biological effects of abnormally high levels of CO in the atmosphere have been intensively studied (4), and research is continuing in this area to more specifically define the effects of CO concentrations encountered under various road traffic situations.

A series of research contracts was awarded to Stanford Research Institute by the Coordinating Research Council and the National Air Pollution Control Administration (later transferred to the Environmental Protection Agency) to provide insight into the fate of atmospheric CO. The objective was to investigate the biosphere as a possible sink for atmospheric CO. Research conducted under the initial contract by Mrs. Elaine Levy (14) showed that nonsterile soil depleted CO from test atmospheres, whereas steam-sterilized soil did not, suggesting a role for soil or soil microorganisms as a sink. Tests regarding uptake of CO by certain large marine algae were inconclusive.

The research described in this report was conducted under a second contract, and was designed as an extension of Mrs. Levy's work. Objectives were to:

Measure CO uptake by soils from different locations.

Determine whether CO uptake by the soil was mediated by a physical or biological mechanism.

Determine which organisms, if any, were responsible for CO uptake.

Determine the role of higher plants as a CO sink.

Determine effects of selected environmental conditions on CO uptake by soil.

Some of the results of this research have been published (8) and an additional paper is in preparation.

II BACKGROUND

Concentrations of CO in the ambient atmosphere appear seldom to exceed 1 part per million (ppm). Swinnerton et al. (22) recorded concentrations over the Atlantic Ocean between Chesapeake Bay and Puerto Rico that ranged between 0.075 and 0.44 ppm. Within the Potomac River-Chesapeake Bay area, concentrations decreased with increasing distance from urban areas, but increased over the large ocean mass. Robinson and Robbins (19) found values over the Pacific Ocean ranging from 0.04 ppm at latitude of 50° S to 0.2 ppm at 40° N. The higher values for the northern hemisphere were attributed to air pollution sources in this hemisphere. Concentrations of CO at ground level at Point Barrow, Alaska, averaged 0.09 ppm through an 11-day sampling period in September (2). Seiler and Junge (20), in a study of the global tropospheric distribution of CO, found the average for the northern hemisphere to be 0.1-0.15 ppm. Robbins et al. (18) measured 0.3-0.9 ppm of CO at ground level in Greenland, 0.8 ppm at one spot on the north coast of California, 0.03-0.3 ppm at Crater Lake, Oregon, and concentrations up to 0.8 ppm at Patrick Point, California. They considered the average ambient concentration of CO in the Northern Hemisphere to be approximately 0.05 ppm.

Both man-made and natural sources contribute to ambient CO levels. Because the largest single source of anthropogenic CO is the automobile, CO levels over large urban centers always exceed those found in remote regions and can be observed to rise and fall with the intensity of motor vehicle traffic. During a recent study in Los Angeles, the CO level at one station along the Harbor Freeway measured 3 ppm at 4:00 a.m., when traffic intensity was the lowest, and 15 ppm at 8:30 a.m. during the morning rush hour (25). Similar patterns are evident in any large metropolitan area. During prolonged periods of air stagnation, CO levels in Los Angeles have exceeded 30 ppm for an 8-hour period. In London, CO concentrations at street level on a calm day have reached 360 ppm.

Robbins et al. (18) have estimated that the worldwide production of CO by man exceeds 200 million metric tons each year. According to Jaffe (9), over 90% of the CO liberated by man in the United States is due to the burning of gasoline by motor vehicles. There are also numerous natural sources of CO. The extent to which these may contribute to the total ambient picture is not yet understood, but evidence is accumulating that indicates that CO produced in ocean waters is a prime contributor to ambient levels. Swinnerton et al. (22) observed that CO content in Atlantic ocean waters was greater than that in the Potomac River and Chesapeake Bay. Moreover, equilibrium for CO between the sea water and the atmosphere was not observed, in that the net gas transport was from the sea to the air. Seiler and Junge (20) found CO concentrations in surface waters of the Atlantic to be 10-40 times higher than would be expected for equilibrium values calculated on the basis of atmospheric concentrations. Swinnerton et al. (23) also found the Atlantic waters to be supersaturated with CO, and recognized the ocean as a significant natural source that may produce an amount equivalent to 5% of that liberated by man. In further studies (29), a bacteria-free culture of the ultradiatom Chaetoceros galvestonensis produced over 5 times as much CO as the illuminated sterile controls although CO production in sea water could also be mediated by a photochemical reaction involving only dissolved organic carbon under sterile conditions. Some CO production was also observed in the dark, however. Production of CO by other marine species such as brown algae (3,16) and the vertically migrating siphonophore, Nanomia bijuga (17), has also been noted. Formation of CO by cucumber seedlings growing in the dark under 5% O_2-95% A atmosphere was noticed by Siegel et al. (21). Euphorbia clandestina produced 200 mg of CO during a 3-month test period within a 16-liter experimental atmosphere containing initially 0.09% O_2, 0.24% CO_2, 1.4% argon, and N_2 to give P = 0.1 atm. CO was also produced at reduced O_2 levels by germinating seeds of rye, pea, cucumber, turnip, and lettuce, but not by seeds of corn, bean, and tomato.

Catabolism of haem-like compounds is known to result in CO production within living or autolyzing systems (4). Likewise, Wilks (28) showed

that macerated tissues of several green plants, most notably alfalfa leaves, evolved CO. Westlake et al. (27) found that the degradation of flavonoids such as rutin by certain fungi, including Aspergillus spp. and Penicillium spp., resulted in CO formation. Hence the process of death and decay in Nature can be suspected of being a prime natural source of CO.

The residence time of CO in the atmosphere has been variously estimated. The most recent calculations range from 0.1 to 0.3 year (6,15,26). This relatively short residence time is circumstantial evidence that one or more sinks for CO exist. Jaffe (9) has suggested several possible sinks, including various elements of the biosphere and atmospheric reactions. Seiler and Junge (20), noting the rapid decrease of CO above the tropopause, considered the stratosphere as a major sink for CO due to its oxidation there by OH, H_2O_2 and HO_2 radicals. A steady-state model of the lower atmosphere constructed by Levy (15) predicted concentrations of hydroxyl and hydroperoxyl radicals sufficient to limit CO residence time in the atmosphere to 0.2 years. Laboratory experiments by Dimitriades and Whisman (6) simulated lower atmospheric conditions in a 50-liter reaction flask, under which CO was oxidized to CO_2 at an approximate rate of 0.05% per hour, equivalent to a natural residence time of approximately 0.3 years. Conversely, evidence also exists that the atmosphere is a natural source of CO as well as a sink. Swinnerton et al. (24) have found that raindrops may show up a 200-fold supersaturation with CO in respect to attendant atmosphere, and postulate that the source of CO in this instance is in rain-forming clouds. They suggest, as a plausible CO-formation mechanism, the photo-oxidation of dissolved organic matter in the rain water or the dissociation of CO_2 by electric discharges in storm clouds.

That certain elements of the biosphere are involved in turnover of CO has been known for some years. Anaerobic methane bacteria are known to oxidize CO to CO_2 in the absence of H_2, or to reduce CO directly to methane in the presence of H_2 (12). Cell-free extracts of Desulfovibrio desulfuricans also oxidize CO to CO_2 in the presence of sulfite (30). In the 1930s, Jones and Scott (10) reported that certain bacteria present

in sealed coal mines were capable of removing CO from the mine atmosphere. Kistner (11) found evidence that the oxidation of CO by the bacterium Hydrogenomonas carboxydovorans was mediated via an adaptive enzyme system, and that the ability to transform CO could be lost following a 24-hour period of culture on organic media lacking a CO atmosphere.

In regard to higher plants, Krall and Tolbert (13) found that cut barley leaves took up CO at a low rate, but that the mechanism was definitely light-dependent and resulted in production of photosynthetic cycle intermediates and serine. Daly (5) observed an increase in the respiration of wild plum leaf tissue subjected to CO atmospheres in the dark. Hill (7) was unable to demonstrate CO uptake by alfalfa.

The above literature, although indicative that carbon monoxide is undoubtedly involved in various biological mechanisms and atmospheric reactions that may influence its concentration and residence time in the atmosphere, does not make clear the role of the biosphere in reducing atmospheric CO. Quantitative data are needed before the biosphere can be considered as a natural sink for carbon monoxide. This report presents data relating to the action of the soil and soil microorganisms in removing carbon monoxide from experimental atmospheres.

III SUMMARY AND CONCLUSIONS

This report describes studies conducted to determine if soils and vegetation could serve as a sink for atmospheric carbon monoxide. This was accomplished by exposing various soils and plant samples to experimental atmospheres containing carbon monoxide. The results show:

1. Higher plants tested had no detectable capacity for decreasing carbon monoxide levels in the atmosphere surrounding them.

2. Soils from a wide range of ecotypes depleted carbon monoxide from atmospheres above them at rates averaging 8.44 mg/hr/m^2 of soil surface. This rate indicates that soils could be a major sink for atmospheric carbon monoxide.

3. The depletion of carbon monoxide by soils was sensitive to temperature, with a maximum depletion rate at 30°C and a Q_{10} of 3-6. Soils sterilized by steam, antibiotics (mixture of cycloheximide, streptomycin, and erythromycin) or salt lost the capacity. However, steam-sterilized soil reinoculated with a small amount of nonsterile soil slowly regained the capacity. Anaerobic conditions totally inhibited the process and there was a slight specificity for a depletion of the lighter isotopes of carbon monoxide. All these facts lead to the conclusion that the process was biological in nature.

4. Constant and prolonged exposure of soil to carbon monoxide reduced the soil's microflora population and also reduced the soil's capacity to deplete carbon monoxide from the atmosphere.

5. Among the bacteria and fungi isolated from soils, 14 fungi (4 strains of Penicillium digitatum, P. restrictum, 2 strains of Aspergillus fumigatus, A. niger, A. fisheri, A. cervinus, 2 strains of Haplosporangium parvum, Mucor hiemalis, Mortierella

vesiculata) were found to possess the capacity in pure culture to deplete carbon monoxide from atmospheres above them.

6. Preliminary experiments with C^{14}-labeled carbon monoxide indicated that soils oxidize carbon monoxide to carbon dioxide.

IV METHODS AND MATERIALS

Test Systems

Plastic Atmospheric Chambers

Plastic atmospheric chambers (PACs) containing soil and experimental atmospheres constituted the major test system. Each chamber had a volume of 11 liters when closed and sealed. Ports in the top and ends of the PACs provided for the introduction of the test atmosphere and its circulation and sampling during the tests. Circulation of the test atmosphere was accomplished by a peristaltic pump. The PACs were tested for leaks prior to use.

When necessary, the PACs were sterilized by submerging them in 10% hypochlorite solution for 10 minutes. They were then air-dried on the "clean" benches. Two liters of air-dried soil, an amount found to give near optimum uptake of CO (Fig. 1), was placed into the PAC and brought to 10-20% moisture with sterile deionized water. Test soil was incubated in the PAC for 2 weeks at room temperature prior to testing. The PACs were also used to aseptically grow seedlings of higher plants for testing.

Prior to testing, the lids of the PAC were sealed and the PAC was connected to the peristaltic pump. The test atmosphere was then introduced into the PAC by purging with 100 ppm CO in balance air at 2 cfm for 5-10 minutes. The system was then closed and a 1-ml gas sample was immediately withdrawn via a gas-tight syringe and analyzed for CO content, providing the "zero time" reading for the test. Additional samples were withdrawn periodically to determine the rate at which CO was being depleted from the test atmosphere.

In the field, PACs were positioned upside down over the soil and then pressed into the soil to a depth of 2.5 inches (a depth that achieved approximately the same soil:atmosphere ratio as that in the PACs in the lab). Soil was then pressed down against the outside edge of the

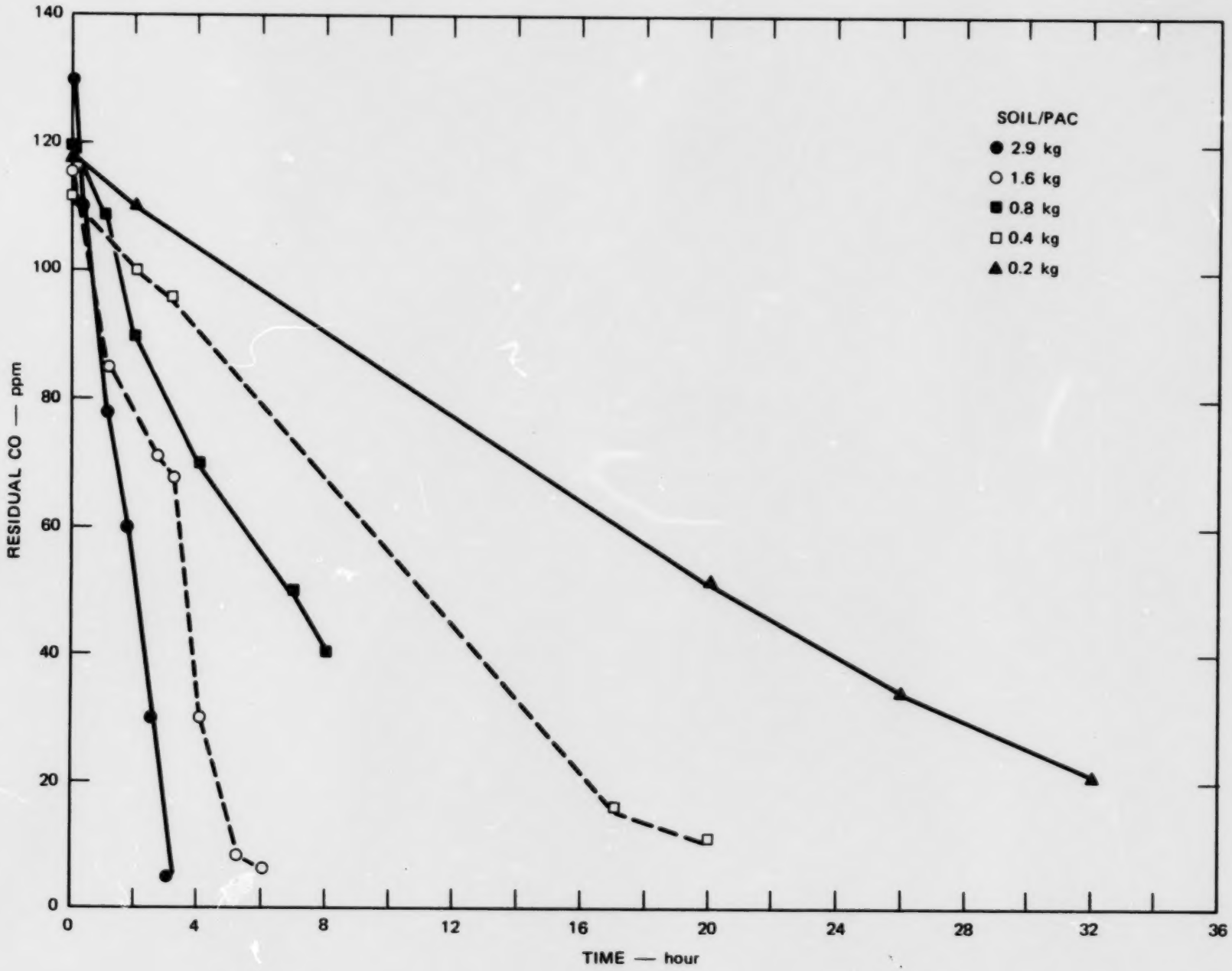

FIGURE 1 INFLUENCE OF SOIL VOLUME IN PAC ON CO UPTAKE

PAC. The procedure for gassing and sampling was the same as for PACs in the lab except that no peristaltic pump was attached in the field experiment.

Flasks

For studies requiring numerous replicates or exact temperature regulation, small 250- and 500-ml filter flasks were used to contain the test material. The flasks were stoppered with rubber stoppers fitted for gassing and sampling. The atmosphere was not circulated in flasks.

Microorganisms isolated from soils were tested in 250-ml filter flasks for their CO uptake capacity in pure culture. The microorganisms were grown on 10 g of vermiculite moistened with potato dextrose broth (20 ml for bacteria, 30 ml for fungi). The inoculated flasks were incubated 2 weeks prior to testing to allow sufficient growth to occur. The flasks were then flushed with 100 ppm CO in balance air and sealed; a 1-ml initial sample was taken for analysis. After 4 hours, a final sample was taken and analyzed. During the test period, the flasks were maintained in an incubator water bath at 25°C.

Clean Benches

Two "clean" benches were built to provide a relatively sterile environment for a number of the experiments. The benches were enclosed on three sides and the top. A vertical laminar flow of purified air at 100 cfm was maintained across the top of the benches. The air was purified by passing it through an absolute filter (99.97% efficient for 0.3-μ particles). Lighting was provided by twelve 40-w cool-white fluorescent tubes mounted 30 inches above the working surface. A 12-hour diurnal light cycle was maintained. The inside walls of the clean bench were periodically surface-sterilized using a 10% hypochlorite solution. The benches were maintained in a separate air-conditioned room to minimize contamination and drafts and to help regulate the temperature. Nutrient agar plates left open on the benches for 30 minutes picked up no contamination.

Gas Analysis Apparatus

The analytical technique for the measurement of CO involved its catalytic reduction to methane followed by flame-ionization detection of the methane, as used and described by E. Levy (14).

Test Materials

Soils

The potting soil used in the majority of these studies was a mixture of sandy loam (95%) and Canadian sphagnum peat moss (5%). Natural soils were collected at selected locations in California, Florida, and Hawaii. The gross duff surface cover was removed from the soil surface prior to collection. These soils were air-dried at 80°F, stored in sealed containers, and held for testing. For tests, soil aliquots were adjusted to the required moisture level and incubated for 2 weeks at room temperature prior to treatment and testing.

Higher Plants

The plant species tested were grown from seed obtained from commercial sources in the case of trees, crops and ornamentals, from the Rancho Santa Ana Botanical Gardens, Pomona, California, for the desert plants, and from field collections for the common weeds. To attain aseptic plants, the seeds were surface-sterilized in a 10% hypochlorite solution for 6 minutes, rinsed with sterile distilled water four times, air-dried on the "clean" bench, and planted in steam-sterilized potting soil in the PACs. From 100 to 500 seeds, depending on the species, were planted in each PAC to provide a dense stand of seedlings. The seedlings were raised in the PACs on the "clean" benches with a 12-hour light cycle.

Microorganisms

All of the microorganisms tested in this study were isolated from the soils collected in California. Two weeks prior to isolation, soil samples were brought to 10% moisture with sterile deionized water and incubated at room temperature. Fifty grams of this incubated soil was then blended with 450 ml of 1/4-strength sterile Ringer's solution. This soil solution was allowed to settle for 30 minutes; 1-ml aliquots were

then withdrawn in triplicate for serial dilution in sterile soil extract to 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} dilution liters. A 1-ml aliquot of each of the dilutions was plated on 10 different nutrient agars (Czopek's Dox + Actidione, Littman-Oxgall, Topping's Medium, Taylor's Soil Extract Medium, Wort, Heart Infusion, Lockhead's Soil Extract Medium, W L Differential Medium, Basal-Amino Acid-Vitamin Tryptophan Medium (BAVT), and Algae Simple Salts Medium. Each dilution was replicated on each medium three times. After 3-5 days of incubation at 29°C, the various forms of growth present on the plates were segregated on the basis of colony characteristics and isolated on the same medium. For comparisons, the individual isolates were all transferred to a common medium (BAVT agar) and the organisms were grouped according to gross morphological characteristics.

Soil Treatments

Steam Sterilization

Two liters of air-dried soil was placed into 8 x 16 x 2-inch Pyrex dishes enclosed in paper bags, and then autoclaved for at least 30 minutes at 121°C and 15 psi. After being cooled, the soil was dumped into a sterilized PAC, moistened to 10% moisture with sterile deionized water, and tested for its CO uptake capacity.

Antibiotic and Saline Treatments

Potting soil samples (200 g) were placed in 500-ml filter flasks and treated with 50 ml each of erythromycin (500 ppm), cycloheximide (500 ppm), streptomycin (500 ppm) or 10% saline (NaCl) solution. The flasks were allowed to incubate 4 days prior to testing.

In further experiments, a 2-liter (air-dried) sample of potting soil was thoroughly mixed by agitation inside a plastic bag with 150 ml of a solution containing 1000 ppm cycloheximide, 510 ppm streptomycin and 870 ppm erythromycin. The soil was then placed into a PAC, allowed to air-dry on the "clean" bench, again drenched with 150 ml of the antibiotic solution, allowed to dry again, drenched with 150 ml, and tested for its CO uptake capacity.

Anaerobic Treatment

Potting soil (100 g) was moistened to 10% and incubated for one week in a 250-ml filter flask. The soil was then incubated for another week under an N_2 atmosphere, which was renewed daily to ensure anaerobic conditions. Ability of the soil to remove CO from the atmosphere was then determined using a test atmosphere composed of 70 ppm CO in N_2 at 25°C.

Inoculation of Sterile Soil

Two liters of steam-sterilized potting soil was placed in a sterile PAC on the "clean" bench, adjusted to 10% moisture content with sterile deionized water, and inoculated with 1 g of nonsterile potting soil. The soil in this PAC was tested daily for its CO uptake capacity for a period of 35 days.

Temperature Treatment

Samples (100 g each) of potting soil were placed into 250 vacuum filter flasks, with 6 replicates per treatment. The soils were brought to 10% moisture and allowed to incubate at room temperature for one week. The flasks were then placed in a water bath in a reach-in incubator. The test temperature was maintained by both the water bath and incubator at $\pm$ 1°C. The flasks were allowed to equilibrate for 30 minutes. They were then gassed, and one flask was sampled in rotation every 10 minutes or longer, depending on the rate of CO uptake. In several of the tests at different temperatures, flasks containing steam-sterilized soil prepared as above were added as controls.

Light-Dark Treatment

Potting soil (200 g, air-dried) was placed into six 500-ml filter flasks, brought to 10% moisture, and incubated for one week. Three of the flasks were then wrapped in tin foil to darken them. The flasks were placed into a water bath in a reach-in incubator (both holding a temperature of 25 $\pm$ 1°C). The lights in the incubator produced approximately 2700 foot-candles of light. The flasks were gassed with 100 ppm CO in balanced air and tested for their uptake capacity.

Isotope Fractionation Technique

Yosemite Valley soil (2 liters, air-dried) was placed in a PAC, brought to 15% moisture, and incubated 2 weeks at room temperature. The PAC was equipped with extra ports to provide for transfer of atmosphere samples into vacuum bottles and for balloons, which inflated within the PAC as large samples were withdrawn by the vacuum bottles. Samples (0.5 to 1 liter) of the atmosphere in the PAC were taken at 1000 ppm CO (initial concentration), 500 ppm CO (50% concentration), 200 ppm (20% concentration), 100 ppm CO (initial concentration), and 50 ppm CO (50% concentration). The sample bottles were then sent to Mr. Charles Stevens at Argonne Laboratories for mass spectral analysis of the CO.

Preliminary Isotope Studies

Yosemite Valley soil was ground in a ball-mill to ensure a uniform texture for sampling. The soil was brought to 15% moisture and incubated for two weeks at room temperature. Samples (30 g) of this soil were placed into 125-ml filter flasks. The flasks were wrapped with tin foil to darken them and placed in a water bath at 25°C. Carbon monoxide-^{14}C (sp. act. 4.5 mCi/mM) was injected into the flasks to bring the starting concentration to approximately 100 ppm CO. A sterile flask with no soil was used as a control. After varying periods of exposure to isotopically labeled CO (30, 60, 100 and 300 minutes), when the concentration of CO as measured by GLC was 66 ppm, 35 ppm, 0 ppm, and 0 ppm, respectively, the atmosphere of the flask was analyzed. The control flask without soil was used for the zero time-100 ppm CO reading. For analysis, the entire atmosphere of the flask was expelled using air as a carrier. The expelled gas was passed through a bubbler containing 8 ml of 1 M hyamin hydroxide in methanol to collect the CO_2. The gases were then passed through a column of hopcalite to convert the remaining CO to CO_2 and through another bubbler with 8 ml of hyamine hydroxide to collect the CO_2 from the CO.

Samples of the hyamine hydroxide (0.8 ml) and soil samples from the flasks (0.3 g) were placed into scintillation vials with 15 ml of the counting cocktail (Aqual-Sol, New England Nuclear). The radioactivity

of each vial was determined on a Beckman LS 100 liquid scintillation counter.

Prolonged Exposure to CO

Soil from Lake Arrowhead was brought to 15% moisture and incubated for 2 weeks at room temperature. Then 50-g samples were placed into eight 250-ml filter flasks. Two of the flasks were sacrificed immediately, and the microorganisms in that soil were isolated according to the previously described technique. The remaining flasks were placed in a water bath at 25°C, and premoistened sterile air (passed through a Millipore filter) was continuously flushed through three of the flasks at ~0.5 cfm. The other three flasks had premoistened sterile air plus 50 ppm CO flushed through them at ~0.5 cfm. The CO uptake capacity of the flasks was determined at 0, 5, 12, 34 and 41 days of exposure. The flasks continuously gassed with air were flushed with 50 ppm CO in air for 8 minutes at 6 cfm, and were then sealed and monitored. The flasks gassed with CO in air were sealed and monitored. Immediately on cessation of monitoring, the flasks were returned to the continuous gassing regime. At the end of the prolonged exposure (41 days), samples of soil from the CO-exposed flask and the air control flask were analyzed for their microbial populations.

V RESULTS

Higher Plants

Previous experiments on the uptake of CO by higher plants (14) were inconclusive because the uptake may have been entirely due to the non-sterile soil used as a support medium. In this experiment, steam-sterilized soil was used to grow the surface-sterilized seed in a sterile environment. Due to limitations on space imposed by the sterilization procedures, only a few species could be tested (Table I). An attempt was made to test species of a wide variety of plant types. However, a number of species (e.g., Phaseolus vulgaris) were not adaptable to the sterilization procedures. None of the plants tested showed the slightest capacity for CO uptake during a standard test period of 3-4 hours, or even for a 24-hour test period.

Potting Soil and Natural Soil

As a result of previous experimentation (14), it was suspected that soil might serve as a sink for atmospheric CO. To test this hypothesis, potting soil was exposed to 100 ppm CO at 28°C in the PACs. This experiment was repeated numerous times, with virtually identical results. Figure 2 shows the results of one typical experiment. As can be seen, the depletion rate of CO in the PAC is nearly linear for the test period of 3 hours. The rate of uptake calculated from this figure is 5.54 mg CO/hr/m^2. Studies with concentrations of CO up to 1,000 ppm were also characterized by a linear rate of approximately 6 mg CO/hr/m^2. Control PACs containing no soil showed no detectable decrease in concentrations during a 4-hour period.

The relationship of the laboratory soil-PAC experiments to soil in its natural state was studied by a field experiment. The uptake of CO by soil in the PACs in the lab was very similar to the uptake by soil in situ in the field covered by a PAC (Figure 3). The similarity of

Table I

HIGHER PLANTS TESTED IN ASEPTIC CULTURE
FOR CAPABILITY TO REMOVE CO FROM ATMOSPHERE

Classification	Species	
Coniferous trees	Pinus radiata	Monterey Pine
	Pinus attenuata	Knobcone Pine
Deciduous trees	Cassia phyllodinea	
	Cassia leptocarpa	
Common weeds	Amaranthus albus	Pigweed
	Rumex crispus	Curly dock
	Sorghum halepense	Johnson grass
Crops and ornamentals	Zea mays	Field corn
	Hordeum vulgare	Barley
	Gossypium hirsutum	Cotton
	Cucumis sativus	Cucumber
	Kalanchoe blossfeldiana	Dwarf Tom Thumb
Desert plants	Salvia columbariae	
	Baeria chrysostoma	
	Coreopsis bigelovii	

the rates indicates that the lab results could be used as an approximation of results on soils in the natural state. The slightly lower rate of uptake by the soil *in situ* could be due to a lower temperature in the field. Very similar results were obtained from soil contained in a plastic bag and buried *in situ* for one month prior to testing. The plastic bag was used to ensure a complete seal for the PAC over the soil.

To determine if the uptake of CO by soil was similar for different types of soil, a number of soil samples collected at different sites in California, Hawaii, and Florida were tested (Table II). These soils are listed in their order of decreasing activity. Each soil was tested at least twice and the tests were averaged to calculate uptake rates

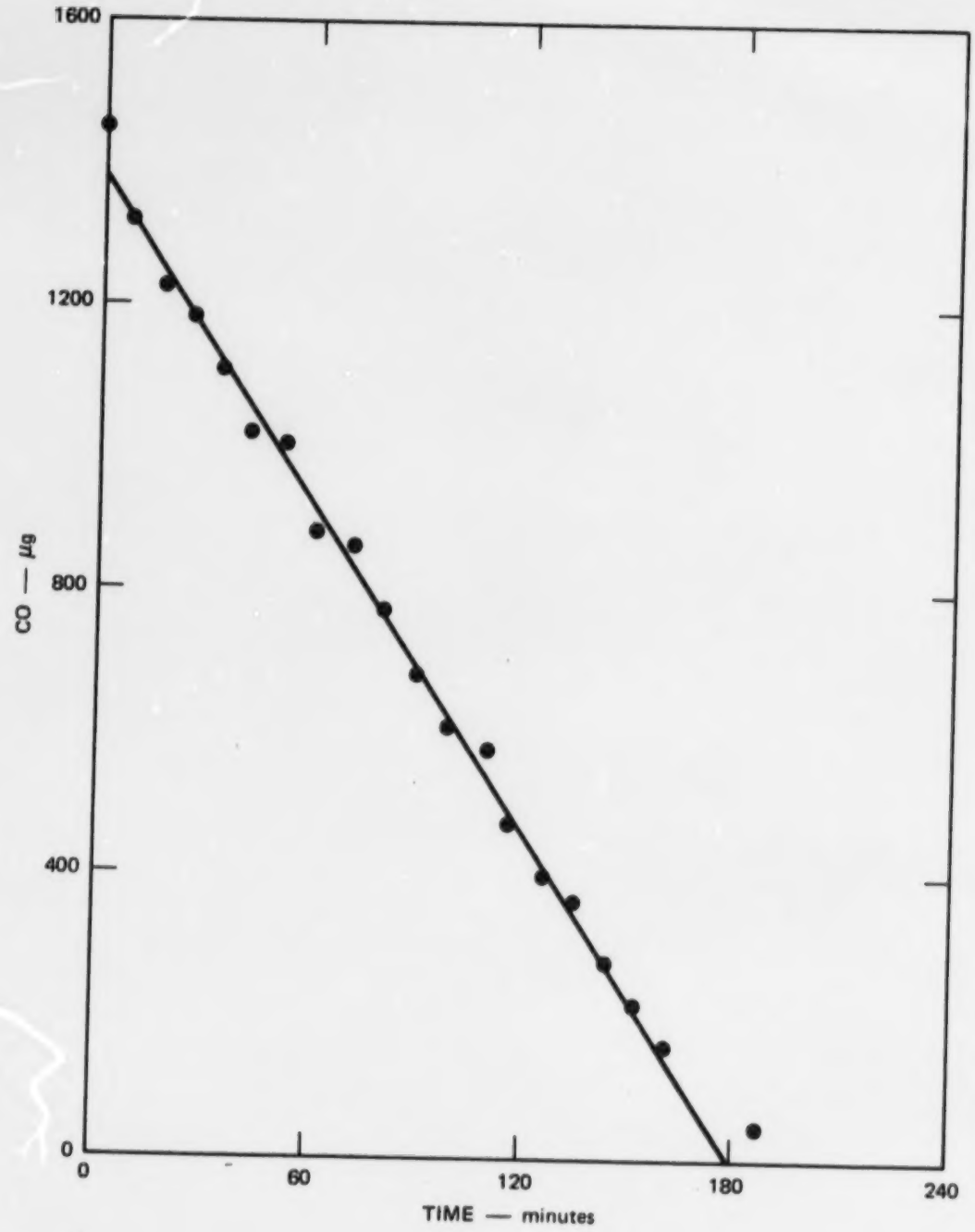

FIGURE 2 REDUCTION OF CO IN TEST ATMOSPHERE WITH TIME BY 2.8 kg OF POTTING SOIL

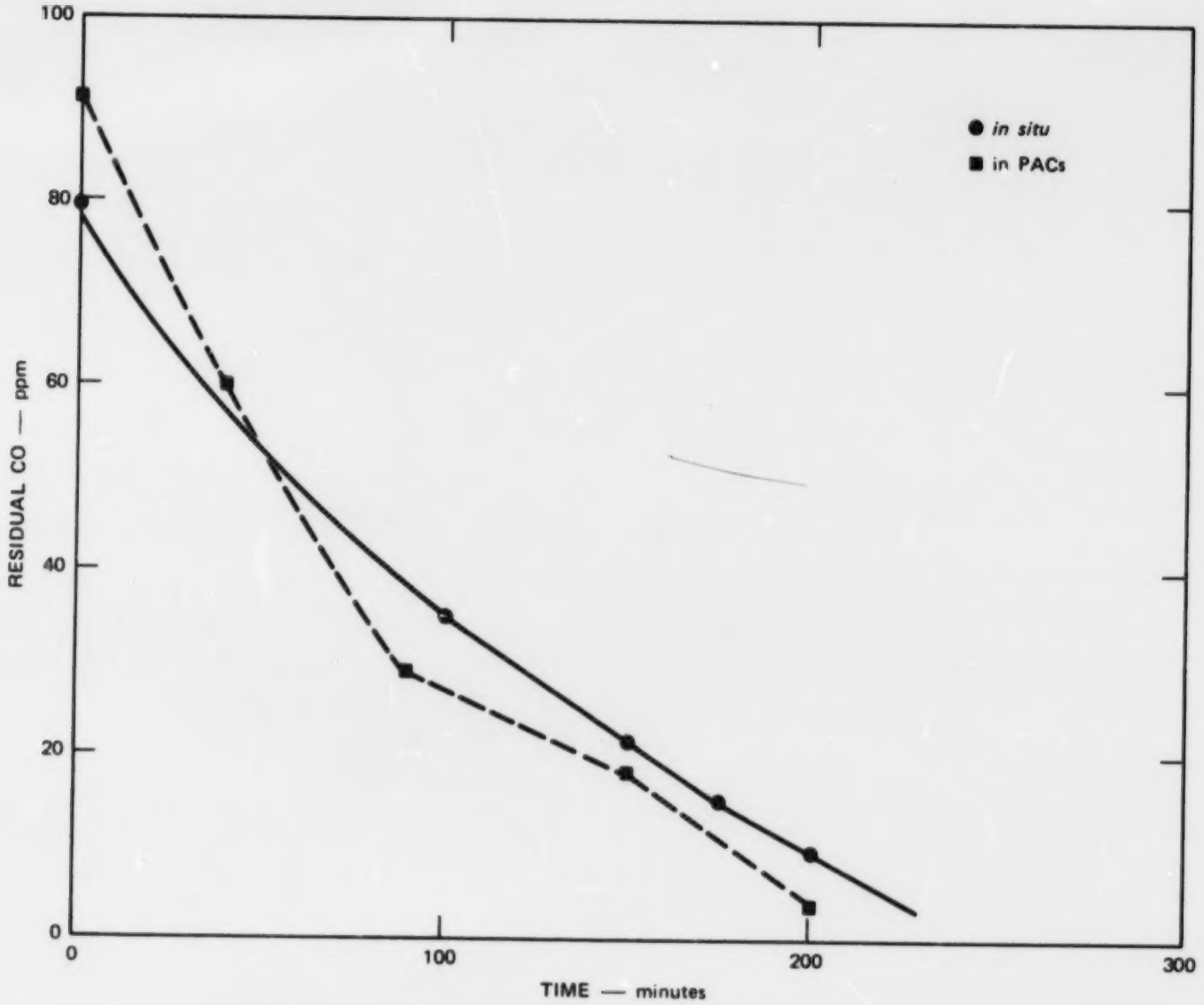

FIGURE 3 UPTAKE OF CO BY SOIL IN PACs AND *IN SITU*. Points represent average of 2 determinations.

Table II

RATE OF REMOVAL OF CO FROM TEST ATMOSPHERES AT 25°C BY VARIOUS SOILS

Location[a] of Soil	Vegetation	pH	Sand: Silt:Clay	% Organic	CO Uptake[b] (mg/hr/m^2)
Eureka-Arcata	Coast redwoods	5.7	53:34:13	25.1	16.99
H. Cowell St. Pk.	Oak	5.3	73:12:15	11.2	15.92
H. Cowell St. Pk.	Coast redwoods	5.7	57:26:17	13.6	14.39
Lake Arrowhead[c]	Ponderosa pine	6.2	65:24:11	17.4	13.89
Redding	Grass-legume pasture	5.1	53:32:15	21.0	11.94
Riverside[c]	Grapefruit[d]	6.6	75:14:11	4.3	11.48
Yosemite Valley	Grass meadow	5.05	49:42:9	20.6	10.52
Kauai, Hawaii	Forest	4.74	58:18:24	22.8	9.90
San Bernardino[c] Freeway	None	7.2	55:30:15	2.2	6.89
Mojave Desert	Chaparral	7.9	79:6:15	2.4	6.46
Woodland	Oak stubble[d]	6.6	33:32:35	2.1	6.23
Riverside (desert)[c]	Chaparral	7.35	85:4:11	1.0	4.31
Yosemite Wall	White fir	5.1	65:18:17	5.7	3.48
Corcoran	Cotton (fallow)[d]	7.1	57:22:21	2.8	3.48
Hanford	Almond[d]	6.95	53:26:21	3.5	2.82
Boynton Beach, Florida	Weeds (fallow)[d]	6.0	86:0:14	1.4	2.65
Oahu, Hawaii	-	4.93	40:26:34	15.3	2.16

a. All soils were collected in California unless otherwise noted.

b. Average rate at end of test period; 2-3 determinations.

c. Locations where high levels of air pollution occur due to combustion of fossil fuels and photochemical smog.

d. Land under cultivation or with recent history of cultivation.

for each. Generally, soils high in organic content and low in pH were found to have the highest rates of CO uptake. Two exceptions to this trend were observed. Soil from Riverside grapefruit orchard was relatively low in organic content but ranked high in CO uptake capacity. The soil from Oahu, which was low in pH and high in organic content, ranked low in CO uptake capacity. No correlation was observed between the ranking of the soil and its prior history of exposure to high levels of natural atmospheric CO.

Effects of Soil Treatments

The depletion of CO over soil could be due to physical processes such as adsorption onto the surface of soil particles, or to biological processes such as oxidation by soil microorganisms. To determine which of these processes was active in CO uptake, a series of experiments were conducted. The first group of these experiments attempted to eliminate the biological capacity of soils so that any physical property that was active could be measured.

Soil that was steam-sterilized by autoclaving showed no capacity for CO uptake (Figure 4). Because autoclaving is a rather drastic treatment that could possibly affect the physical properties of soil as well as sterilize it, other attempts were made to sterilize soil using antibiotics and salt (Figures 5 and 6). Treatment of soil individually with erythromycin, cycloheximide or streptomycin had only partial effects on the uptake of CO, generally reducing the uptake rate by 50% (Figure 5). A 10% saline solution, however, was 100% effective in eliminating the CO uptake capacity of soil (Figure 5). Part of the inactivity of antibiotics could be due to a lack of total treatment of the soil particles because of problems of wettability. When soil was repeatedly drenched with a combination of cycloheximide, streptomycin, and erythromycin, its CO uptake capacity was completely eliminated (Figure 6). Further, it was found that soil held under anaerobic conditions (nitrogen) also had no capacity for CO uptake (Figure 5).

Steam-sterilized soil inoculated with a minute amount of nonsterile soil gradually regained a capacity for CO uptake (Figure 7), presumably

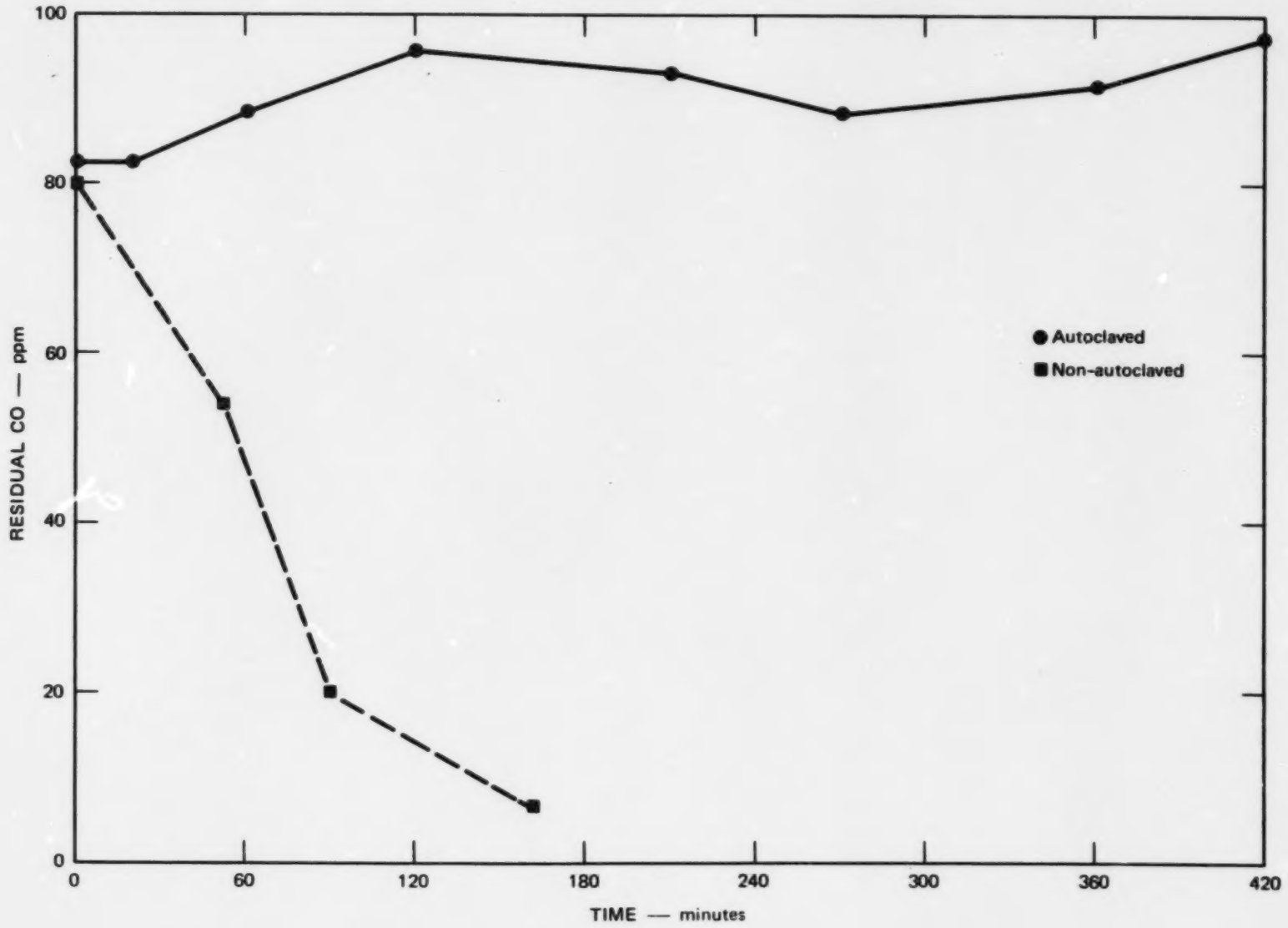

FIGURE 4 CO UPTAKE BY STEAM-STERILIZED VERSUS NONSTERILE SOIL

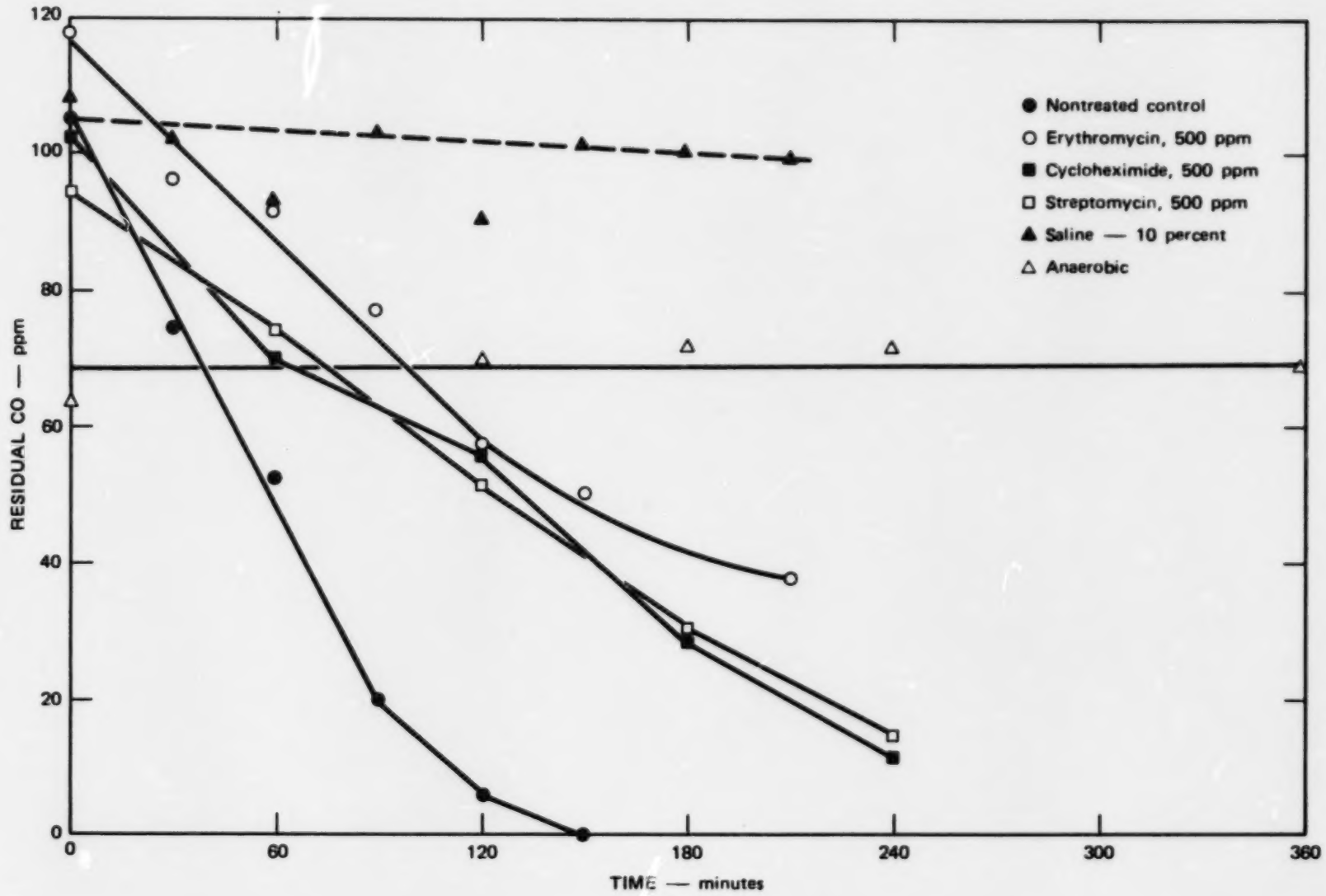

FIGURE 5 EFFECTS OF ANTIBIOTIC, SALINE, AND ANAEROBIC TREATMENTS ON RATE OF CO DEPLETION OVER POTTING SOIL

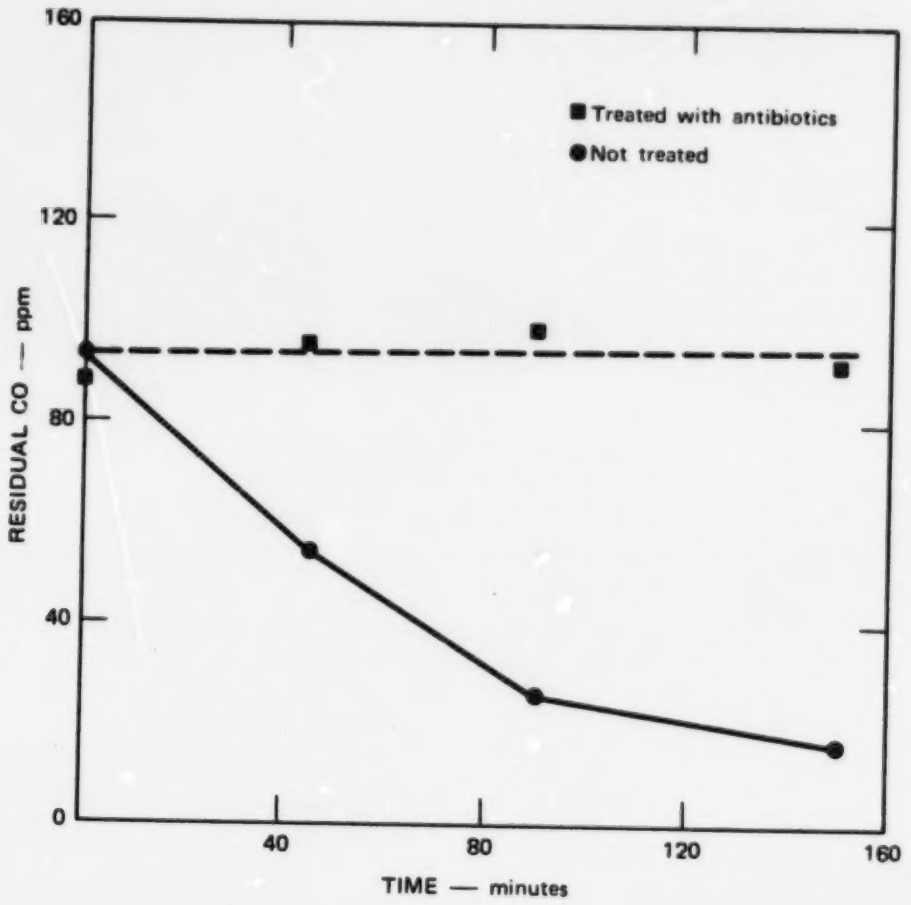

FIGURE 6 EFFECT OF SOIL SATURATION WITH AN ANTIBIOTIC SOLUTION (1000 ppm Cycloheximide, 510 ppm Streptomycin, 870 ppm Erythromycin) ON RATE OF CO DEPLETION

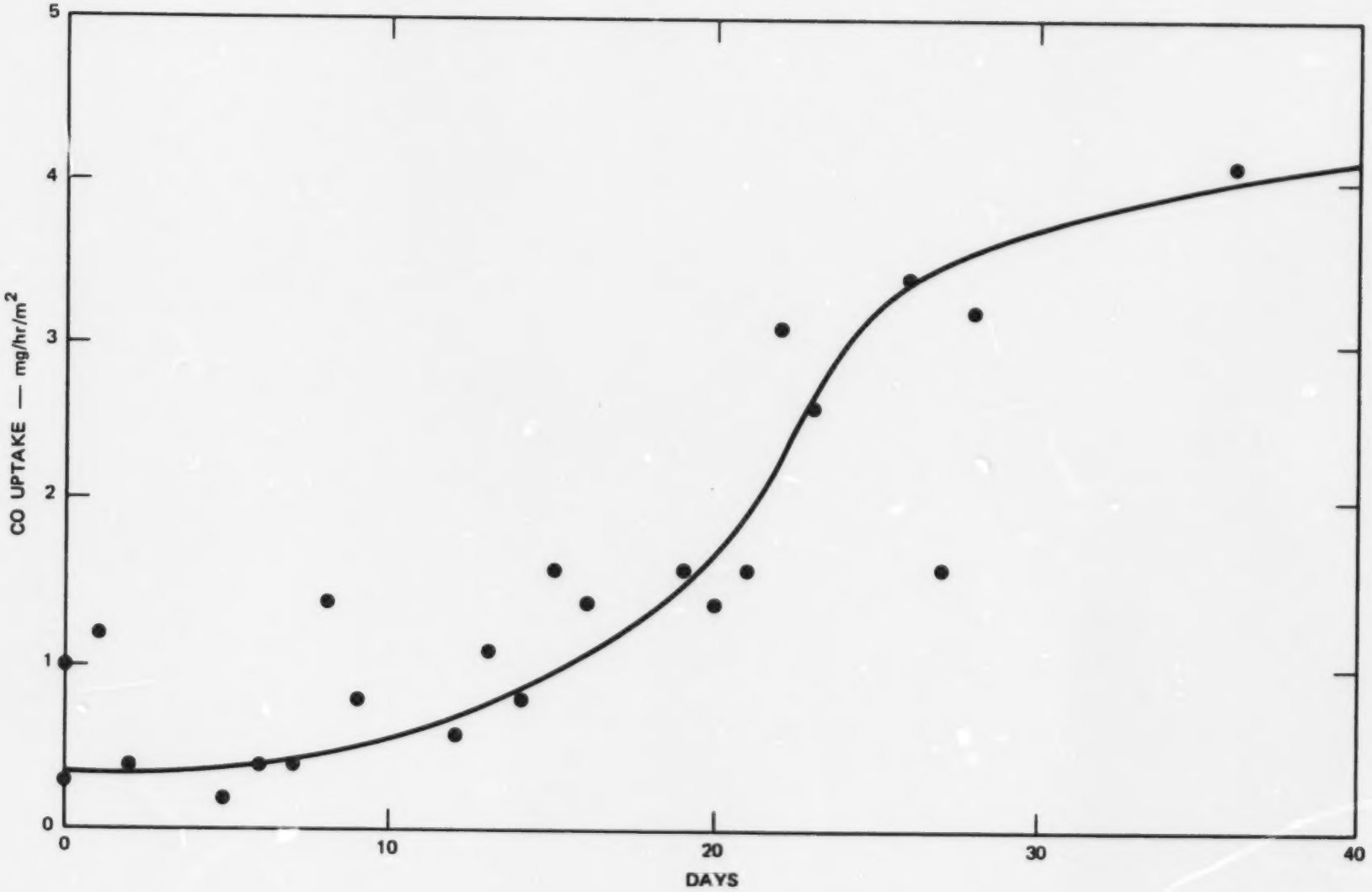

FIGURE 7 CO UPTAKE CAPACITY OF 2.8 kg OF AUTOCLAVED POTTING SOIL WITH TIME FOLLOWING INOCULATION WITH 1 g OF NONSTERILE SOIL

as the soil population of microorganisms was re-established. One month after inoculation, the uptake rate was as great as that of nonsterile control soil.

These experiments suggested rather strongly that aerobic soil microorganisms were responsible for CO uptake. Therefore, the next series of experiments were conducted to further define the biological phenomenon.

The CO uptake rate of soil was found to be very sensitive to temperature changes (Table III). A maximum uptake rate was found at 30°C, with almost no uptake at the extremes of 10 and 50°C. The temperature coefficient (Q_{10}) of this process ranged from 3 to 6 between the temperatures of 15 and 30°C. High Q_{10}s and optimum rates between 20 and 40°C are very characteristic of biological processes. On the other hand, physical processes would increase continuously as temperatures increased and have a Q_{10} in the range of 1-1.4.

Table III

RATE OF CO REMOVAL OVER 100 g
OF POTTING SOIL AT DIFFERENT TEMPERATURES

Temperature (°C)	CO Uptake Rate* (mg/hr/m²)	Test Period (hr)
10	0.30	24
15	0.38	6
20	1.25	5
25	2.38	3
30	3.46	2
35	2.44	2.3
40	1.89	4
45	1.17	4.5
50	0.20	19

*Average rate at end of test period.

The initial experiments with steam-sterilized soil (Figure 4) indicated that treated soils showed a slight evolution of CO. This phenomenon became more pronounced at higher temperatures (Table IV). At 50°C, the rate of CO evolution, 136 μg CO/kg soil/hr, was nearly as great as the uptake of CO would be at its maximum by that much soil. This phenomenon could be observed even when the steam-sterilized soil was stored in an open-air container prior to being tested at a higher temperature.

Table IV

CO EVOLUTION BY AUTOCLAVED SOIL

Incubation Temperature (°C)	CO Evolution Rate (μg CO/kg soil/hr)*	
	Dark	Light
20	3.3	-
40	36	46
50	136	-

*Based on the average of three replicates.

Light had very little effect on the uptake or evolution of CO by soil (Figure 8 and Table IV). The small differences between light and dark were probably due to the 0.5°C higher temperature in the flasks in the light.

Mass spectral analysis of the isotopes of CO remaining at various times during CO depletion by soil shows that there is some isotopic fractionation (Figure 9). Both C^{12} and O^{16} containing CO molecules were removed in preference to those containing C^{13} and O^{18}. This is a consistent trend for biological processes that oxidize organic compounds. However, this is not an absolute proof of biological activity for, in general, the lighter isotopes are more reactive. The differences between samples designated I and II are due to the fact that these samples were drawn from different gas tanks.

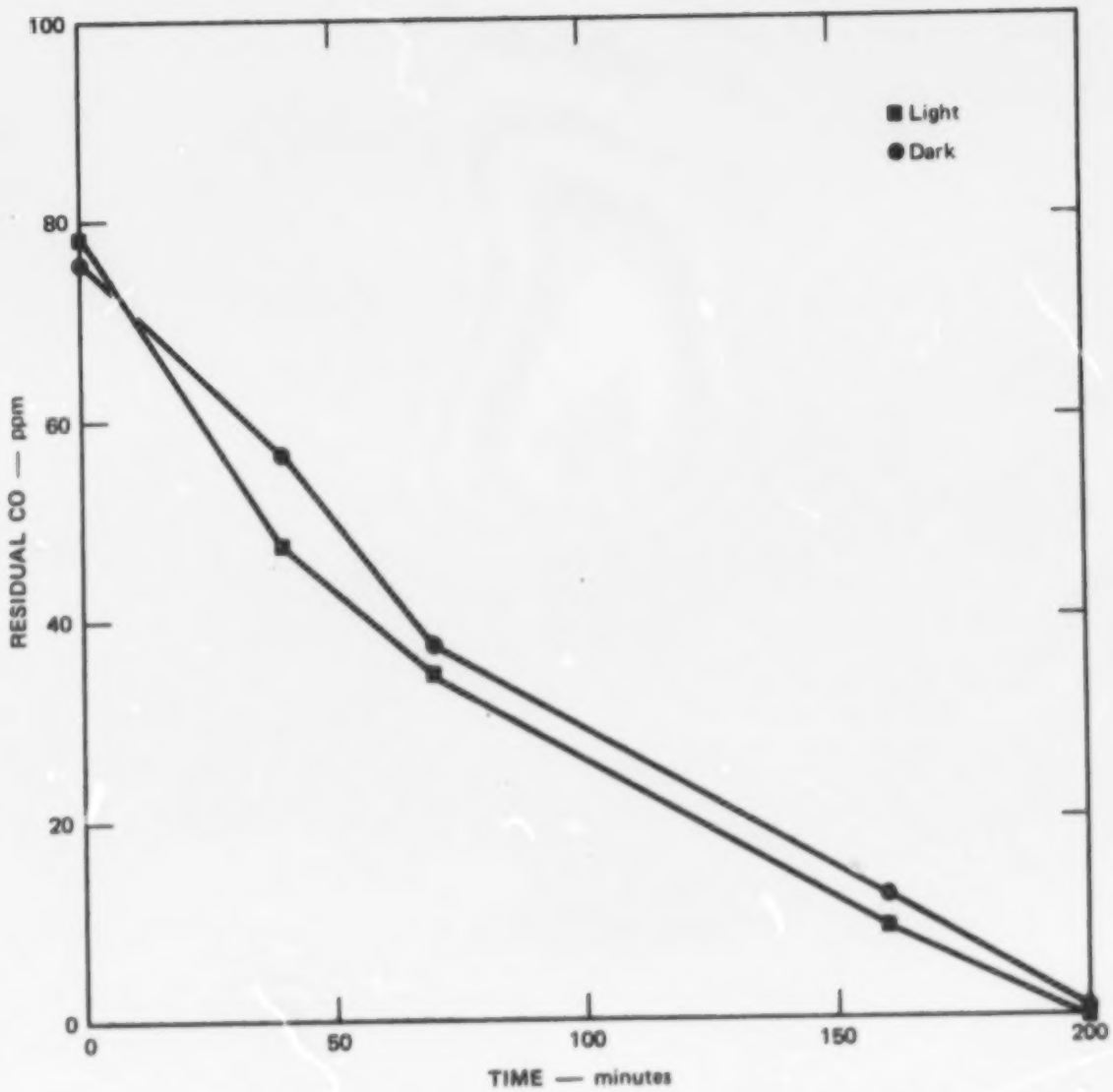

FIGURE 8 INFLUENCE OF LIGHT ON CO UPTAKE BY SOIL

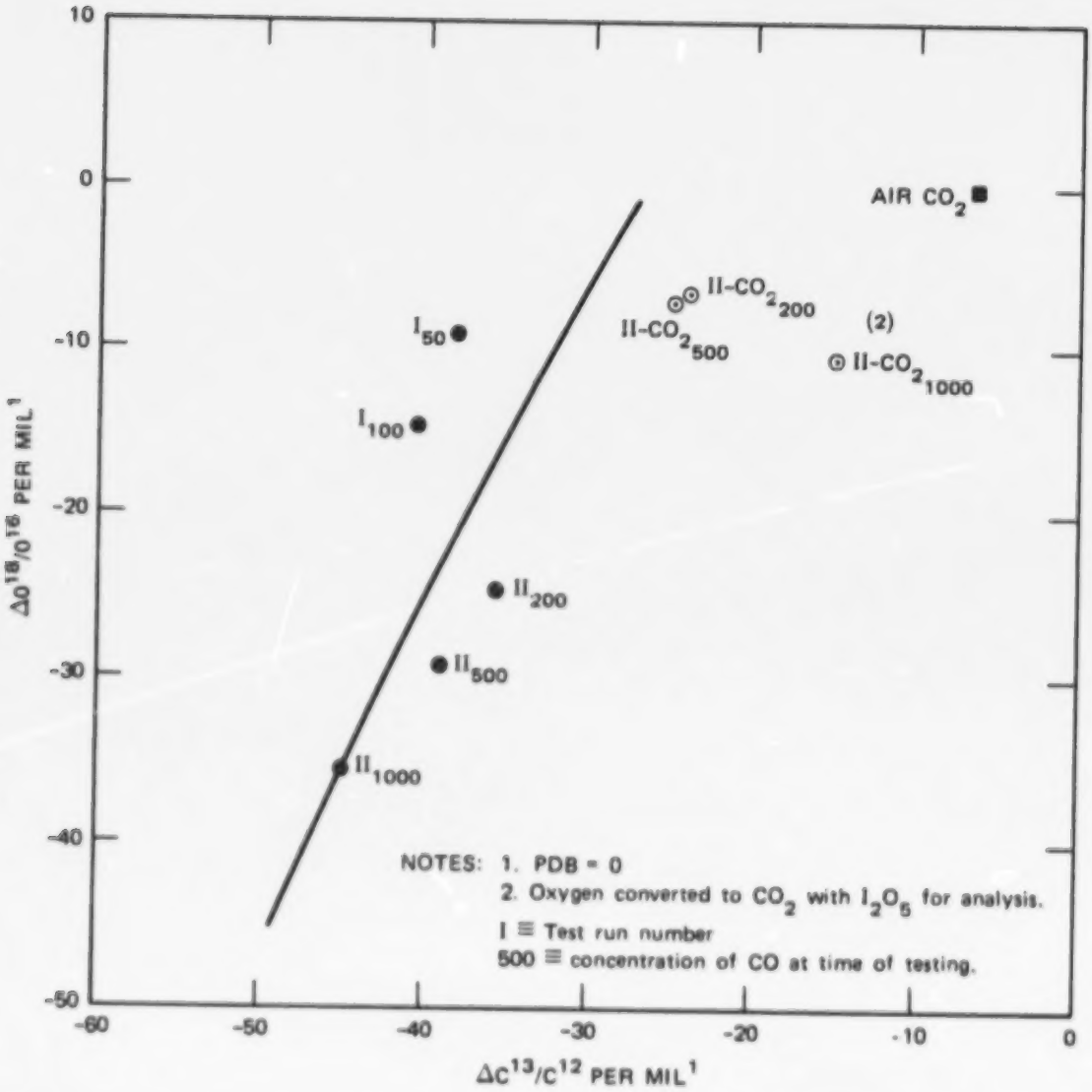

FIGURE 9 ISOTOPIC FRACTIONATION OF CO DURING CO UPTAKE BY SOIL
(Provided by C. M. Stevens)

Soil Microorganisms

Since the uptake of CO by soil was shown in the preceding experiments to be biological, a search for the specific microorganisms involved was conducted. Isolates were made from the Lake Arrowhead soil (40 bacteria and 35 fungi), the Yosemite Valley soil (50 bacteria and 24 fungi), and the Riverside desert soil (48 bacteria and 24 fungi). The fungi active in CO uptake are listed in Table V. The uptake rates listed for these fungi were calculated from the rate over the first

Table V

SOIL FUNGI ACTIVE IN CO UPTAKE

Species	Strain No.[1]	CO Uptake Rate[2] (μg CO/hr/m^2)
Aspergillus fumigatus	1	1.824
Aspergillus fumigatus	2	1.000
Aspergillus niger		1.838
Aspergillus fischeri		1.054
Aspergillus cervinus		1.000
Haplosporangium parvum	1	1.027
Haplosporangium parvum	2	1.684
Mortierella vesiculata		0.568
Mucor hiemalis		2.351
Penicillium digitatum	1	2.292
Penicillium digitatum	2	1.714
Penicillium digitatum	3	2.373
Penicillium digitatum	4	1.768
Penicillium restrictum		0.959

[1] Morphologically different types of the same species.

[2] Calculated from the first test of the second subculture of the organism.

4-hour exposure period. The figures should not be taken as absolute, however, for it was very difficult to standardize the experiment. The organisms all grew at different rates and it was found during retesting that in pure culture, all of these fungi--over a period of 2-3 months and during 3-4 subcultures--lost all capacity for CO uptake. Although no bacteria that could take up CO were found, this does not preclude the possibility that some soil bacteria may be active. The isolation procedure used isolated only a fraction of the soil bacteria, and the testing conditions were not optimum for the growth of a number of bacteria.

Influence of CO on Soil Microbial Population

Soil samples were continuously exposed to atmospheres with and without 50 ppm CO for an extended period (41 days), and the influence on the soil population of microorganisms was studied. The prolonged exposure to CO greatly reduced the capacity of the soil for CO uptake. After 41 days of exposure (Table VI), the soil had only one-fourth the uptake capacity of unexposed soil. The prolonged exposure had little influence on the soil fungal population. However, both the number of species as well as the total number of bacteria were apparently reduced by the exposure (Table VII).

Table VI

EFFECT OF PROLONGED EXPOSURE
TO CO ON CO UPTAKE BY SOIL

Days of Exposure	Rate of CO depletion[1] ($mg/hr/m^2$)	
	Exposed to CO-free air	Exposed to 50 ppm CO in air
0	6.65	7.16
5	6.57	4.54
12	5.35	4.11
35	7.13	2.37
41	6.45	1.46

[1] Rates calculated on an average of two replicates.

Table VII

INFLUENCE OF PROLONGED EXPOSURE
TO CO ON SOIL MICROFLORA

Treatment	Bacteria		Fungi	
	Number of Varieties Isolated	Total No./g soil *	Number of Varieties Isolated	Total No./g soil *
Prolonged exposure to 50 ppm CO	36	2×10^5	79	3.6×10^5
Control kept under CO-free air	53	3.2×10^7	72	2.8×10^5

* Average of two replicates.

Tracing CO Uptake with ^{14}C

Studies on the pathway of CO in the uptake process by soil, using ^{14}C-labeled carbon monoxide (Table VIII), indicate that CO is oxidized to CO_2 and not bound in the soil. The results were highly variable, probably due to the low recovery rates and the low efficiency and non-specificity of hyamine hydroxide for absorbing CO_2. The results do definitely indicate that as the CO decreases, as measured by GLC, radioactivity was still picked up from the gaseous phase by the hyamine hydroxide and no radioactivity was detected in the soil. Only after most of the CO was oxidized, did the label become fixed in the soil. This delayed uptake could be due to CO_2 fixation by the soil microorganisms.

Table VIII

UPTAKE OF ^{14}C-CARBON MONOXIDE BY SOIL

Sample	Replicate	Time of Exposure (min)	Residual CO (ppm)	Radioactivity (cpm)[1]				
				CO_2	CO	Soil	Total	Percent Recovery
Control	A	240	81	19,564	20,851	-	40,415	9.35
	B	240	81	18,580	19,806	-	38,386	8.88
Test	A	30	66	129,301	18,844	0	149,145	31.58
	B	30	66	131,285	19,312	0	150,597	31.89
	A	60	35	33,152	27,004	105	61,206	14.31
	B	60	35	30,243	25,688	48	58,411	13.19
	A	100	0	270,696	31,895	225	302,816	68.66
	B	100	0	275,983	30,512	300	306,795	69.56
	A	300	0	28,569	17,030	270	45,869	9.81
	B	300	0	30,777	19,749	210	50,736	10.85

[1] Counting efficiency 90-93% figures corrected for dilution and background (50 cpm).

VI DISCUSSION

The rate of disappearance of CO from the atmosphere over soils of different ecotypes ranged from 2.16 to 16.99 mg/hr/m^2. The average rate of CO uptake from these soils was 8.44 mg/hr/m^2, which is equivalent to 191.1 metric tons/year/square mile. If it is assumed that this average rate is representative of the average capacity of temperate zone soils, then the total capacity of the soil surface of the conterminal United States (2,977,128 square miles) is estimated to be 569 million metric tons of CO per year. This value is 5.5 times the estimated amount of man-made CO released annually in the United States and is 3 times that released worldwide. This rapid uptake rate is considerably greater than values reported for photochemical atmospheric reactions (6). Soil could be the large sink for CO that is implied by the relatively short residence time (less than one year) estimated in recent studies of the problem (19,26).

The above estimate of soil capacity was based on a limited number of laboratory observations and a large number of assumptions, and is thus only a crude approximation. A more accurate measurement could and should be obtained by an extensive field survey backed by a laboratory study of the relative significance of the field variables. Recent studies (24,29) indicate that there are a number of significant natural sources of CO. Those studies, together with the findings reported here, indicate that a CO turnover cycle surely existed prior to man's recent contributions. More refined knowledge of this cycle would allow for a more precise definition of the fate of the 200 million metric tons of CO produced annually by man. This information would also provide a basis for estimating what influence the ever-larger amounts of CO produced by man have on the natural cycle. A more detailed study of soil as a sink is also justified by a recent study by Abeles et al. (1), which indicates that soil may be a sink for other atmospheric pollutants.

They found that soil took up ethylene, sulfur dioxide, and nitrogen dioxide at rates roughly equivalent to the rate reported here for CO.

There is now sufficient evidence to state that CO uptake by soil is a biological process and that a number of soil fungi contribute significantly to the process. Although no bacteria were found to be significantly active in CO uptake, previous studies (10-12,30) have demonstrated that certain soil bacteria do have this capacity. Failure to find any in this case may be due to inadequacies in the isolation and/or testing techniques, or the CO uptake rates of the active bacteria may simply be too low to be of any significance.

Although the isotope tracer studies involving CO were rudimentary, it would appear that the fungi may not incorporate CO into protein but, instead, may oxidize it to carbon dioxide. The oxidation of CO by microorganisms has been previously described (12,30). Since CO is a potent metabolic inhibitor, such an oxidative capacity could serve as a defense mechanism. It may even be that the CO never enters the cells of these organisms but is oxidized at the cell surface by membrane-bound enzymes.

It would seem that agricultural lands, due to cultural practices, would have a texture and fertility that would provide for optimum microbial growth and, hence, maximum rates of CO uptake. However, the few agricultural soils that were tested generally ranked low in relative CO uptake capacity. The balance of microorganisms could be upset by agricultural practices, or the use of fungicides and other agricultural chemicals could suppress the growth of the active microorganisms and thus account for lower uptake activity.

The lack of CO uptake by any of the higher plants tested agrees with the findings of Hill (7) for a field of alfalfa. Although these negative results do not rule out the possibility that some species of plants could take up CO, at present it appears unlikely that plants contribute significantly as sinks for CO. However, it does appear likely that plants could influence the properties of soil by altering the soil microflora, by changing the porosity of soil, and/or by influencing the gas-diffusion patterns above the soil.

The practical effect of the uptake of CO by soil on lowering local atmospheric CO levels in areas of high air pollution may be limited due to the high atmosphere:soil surface ratio. It would appear more likely that the significance of soil as a CO sink is greater on a regional or global basis than on a local basis.

No correlation was found between a soil's CO uptake rate and the general level of air pollutants in the respective areas in which the soil was collected. Also it was found that prolonged exposures to CO reduced the soil's capacity for uptake. Possibly, soils in areas of high and prolonged levels of atmospheric pollution (e.g., Los Angeles Basin) are being poisoned and are gradually losing their capacity for uptake. On the other hand, perhaps the prolonged exposures may be providing a selective pressure for the evolution of a better CO-oxidizing organism.

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